periods of time, necessitating the maintenance of a nearly equable temperature, and the remaining at rest for many hours, before the top part of the mass becomes sensibly free from suspended portions of the heavier alloy, and the bottom part from similar portions of lighter alloy. The analytical numbers obtained on examining different layers of the compound ingots prepared in the experiments described in the earlier parts of these researches long ago convinced us of this; but, in addition, an actual visible presence of suspended particles of one alloy in the midst of another, even after 8 hours tranquil fusion, may be often observed in the case of silver-lead-zinc and silver-bismuth alloys where the proportions of metals used are such as to form mixtures containing considerable amounts of Ag_4Zn_5: by the aid of a lens, or even with the naked eye, red particles disseminated through a much lighter coloured matrix can often be distinguished on examining the central portions of an ingot that has been filed smooth and bright, and then kept for awhile so as to allow the red tinge to develop.

IV. "On the Structure of Amœboid Protoplasm, with a Comparison between the Nature of the Contractile Process in Amœboid Cells and in Muscular Tissue, and a Suggestion regarding the Mechanism of Ciliary Action." By E. A. Schäfer, F.R.S. Received January 26, 1891.

It has been shown by the researches of numerous histologists, of whom Heitzmann and Frommann, and, in this country, Klein, must be reckoned the pioneers, that the protoplasm of many cells exhibits the appearance of a network containing an apparently homogeneous material in its meshes. The network is known as the *reticulum* or *spongioplasm*, the clear material in its meshes as *enchylema* (Carnoy) or *hyaloplasm*. In many cells it is not difficult to observe this structure even without the addition of reagents, but in amœboid cells such as the white blood corpuscle and the amœba it is less obvious, and its presence has not been generally conceded. Recently, Professor Stricker* has published a photograph of an amœboid white blood corpuscle, taken instantaneously by aid of the electric light, which shows the reticular appearance in quite an unmistakable manner; it must be granted, therefore, that the amœboid white blood corpuscle also has this structure.

Previously to the appearance of Professor Stricker's photograph, I had myself for some time been engaged in investigating the structure of amœboid cells with the aid of photography. Being unprovided

* 'Wiener Medic. Jahrb.,' 1890.

with the appliances necessary for photographing by the electric light, I was unable to obtain instantaneous photographs, and could not photograph the corpuscles while actually living and moving. I accordingly adopted a method of suddenly killing the corpuscles whilst still in the amœboid condition with their pseudopodia extended. It is well known that with most methods which are employed to fix the white blood corpuscles there is time for a contraction of the protoplasm to be produced, so that the pseudopodia are withdrawn and the corpuscle becomes spherical. The method which I have used consists in the instantaneous application of a jet of steam to the surface of the cover-glass. A preparation of blood, preferably from the newt (*Triton cristatus*), is made either in a moist chamber or in the usual way on a glass slide. In a short time the white corpuscles become highly amœboid and throw out pseudopodia, which may spread themselves in a thin layer upon the cover glass in a manner which is perfectly adapted for their being accurately observed. If the steam be now turned on for an instant, the cells are suddenly killed, and remain exactly in the condition in which they happened to be when the heat was applied. They can be examined and photographed thus, or may first be stained by hæmatoxylin, with or without being previously treated with alcohol. In all cases they exhibit the same general structural appearances, and these appearances can even be detected, but with greater difficulty, in the cell whilst still living.

Leaving the nucleus, which beautifully exhibits the karyoplasmic network, out of consideration, the most striking point in all amœboid white corpuscles thus prepared is the contrast between the protoplasm of the body of the cell and that of the pseudopodia. For whilst the former exhibits, according to focus, either a finely punctated or a reticular aspect, and stains decidedly with hæmatoxylin, the pseudopodia exhibit not the faintest trace of structure, and remain almost entirely unstained.

In other words, the protoplasm is composed of two morphologically distinct parts, one which exhibits a reticular arrangement and has an affinity for hæmatoxylin, and another which shows to the best optical appliances no structural arrangement, and is also chemically different, as is shown by its behaviour to staining reagents.

The observation here recorded is not an isolated one. Almost all observers who have given special attention to the matter have failed to detect a reticular structure in pseudopodia, whether of the amœboid cells of higher organisms or of the Rhizopoda. To Bütschli's theory of the structure and activity of protoplasm,* whereby he endeavours to show that the reticular appearance and amœboid phenomena may be explained on the assumption that protoplasm is

* 'Heidelberg Verhandlungen,' 1890; and 'Biologisches Centralblatt,' 1890.

not an actual network with enchylema, but rather a frothy mixture of two dissimilar substances, this absence of all apparent structure in pseudopodia offers an admittedly serious difficulty, which he endeavours to surmount by assuming that the same frothy structure is really present in the pseudopodia as in the body of the cell, but that owing to thinning out it cannot be detected. But apart from the unlikelihood of our not noticing such structure in the pseudopodia if it were really present, since they are especially well adapted for minute observation, the reticular and the homogeneous substances should, according to this assumption, pass gradually the one into the other, for the thinning-off of the pseudopodia is frequently gradual. The contrary is, however, the case. The line of demarcation of the reticular substance is always quite sharp, and does not thin off into the homogeneous substance of the pseudopodia.

Stricker's photograph is also really evidence in the same direction. The corpuscle taken is spherical or nearly so, *i.e.*, is in the contracted condition. It has, however, one small pseudopodium. This is absolutely without structure; it is the spherical part of the cell which shows the reticulum.

It is well known that if white corpuscles (and contracted amœboid cells generally) are artificially stimulated, they are always spherical. The spherical form is, in fact, the contracted condition; it is only in the absence of any obvious source of excitation that the corpuscle throws out pseudopodia. The spherical condition is immediately produced by electrical or mechanical stimuli; no doubt, the constant mechanical stimulation which the cells receive in the circulating blood maintains them in the spherical form which they always exhibit whilst moving within the blood-vessels. Possibly, also, the contact of a foreign particle, causing the contraction and withdrawal of the protoplasm which it touches, and the consequent inception of the particle, is another instance of mechanical stimulation.

Now, in the contracted corpuscle, the whole cell appears reticular, and the reticulation is even better marked, *i.e.*, coarser, than that seen in the spread out corpuscle. The pseudopodial protoplasm or hyaloplasm has, in fact, been withdrawn into the meshes of the framework or spongioplasm.

The protoplasm of such an amœboid cell as the white blood corpuscle may, therefore, be regarded as composed of two distinct substances, spongioplasm and hyaloplasm. Spongioplasm has a reticular or sponge-like arrangement, an affinity for staining fluids, is firmer than the hyaloplasm (but, perhaps, not actually solid), and is, in all probability, highly extensile and elastic. Hyaloplasm, on the other hand, is structureless, has little or no affinity for stains, and is highly labile and fluent. It is by the active flowing of the hyalo-

plasm, not by the contraction of the spongioplasm (as conceived by Carnoy*), that the movements of cells are produced.† Of the two substances, the hyaloplasm is the more active, the spongioplasm the more inert. The spongioplasm forms, in fact, a sort of framework supporting the hyaloplasm, and into which under the influence of stimuli the hyaloplasm becomes wholly withdrawn. To adopt Bruecke's well-known terminology, the hyaloplasm is the *zooid*, the spongioplasm its *œcoid*.

Whether one or other of these two substances is ever wholly absent from the protoplasm of cells is a question which cannot at present be decided. There are cells and unicellular organisms, both animal and vegetable, in which no reticular structure can be made out, and these *may* be formed of hyaloplasm alone. In that case, this must be looked upon as the essential part of protoplasm. So far as amœboid phenomena are concerned, it is certainly so; but whether the chemical changes which occur in many cells are effected by this or by spongioplasm is another question. Certainly, the reticulum is always very well marked in cells in which considerable chemical changes are produced, *e.g.*, gland cells.

The movements within plant cells must also be regarded as due to the flowing of hyaloplasm. It is, indeed, impossible to conceive that the contraction of a reticulum could produce the circulation of the protoplasm which is seen within a cell of *Vallisneria*. How the flowing is produced is an entirely different question, and one which must at present remain unanswered.

If now we compare the structure of protoplasm with that of striated muscle, we find many points of coincidence. As is well known, the muscle columns of the wing muscles of insects ("wing-fibrils" of authors) are divided by transverse partitions (membranes of Krause) into a series of segments (sarcomeres, Muskel-kästchen of Krause), each of which contains a sarcous element or disk of anisotropous sarcous substance (which is really formed of two halves, their junction being often visible as the line of Hensen), and a homogeneous isotropous substance, which in the extended muscle occupies the intervals between the sarcous element and the transverse membrane. As I have elsewhere recently shown,‡ the substance of the sarcous element is penetrated by pores or canals which extend in each half of the element as far as the line or plane of Hensen, and which are occupied by clear substance continuous with the homogeneous substance of the intervals. The substance of the sarcous element stains with hæmatoxylin and similar reagents, while the homogeneous substance of the clear intervals remains unstained. When the

* 'Biologie Cellulaire,' 1886.

† *Cf.* Leydig, 'Zelle u. Gewebe,' Bonn, 1885.

‡ 'Monthly International Journal of Anatomy and Physiology,' vol. 8, 1891.

muscle contracts, the homogeneous substance passes from the intervals into the pores of the sarcous element, and thus enlarges the latter, while the clear intervals are proportionately shortened, so that in extreme contraction they may disappear, and the swollen and bulged sarcous element may almost abut against the tranverse membranes. On the other hand, when the contraction passes off, and the muscle becomes extended, the homogeneous substance passes out of the pores of the sarcous element into the clear intervals; the latter become manifest, and the sarcous element proportionately diminished in bulk. It is hardly possible that the resemblance of these changes to those which occur in the protoplasm of an amœboid cell is merely accidental—difficult not to believe that the perforated sarcous substance is the spongioplasmic "œcoid," the clear labile substance the hyaloplasmic "zooid."

This conception of the structure and mode of activity of the amœboid cell and of muscle, whilst bringing them under exactly the same category, and thus tending to simplify our ideas regarding contractile phenomena, may also serve to aid in the elucidation of certain questions in connection with those phenomena which have long presented difficulties to the physiologist and pharmacologist. For example, with regard to the movements of amœboid cells, the question has been frequently discussed, and never satisfactorily answered, whether we are to regard the withdrawal of the pseudopodia into the body of the cell as the condition of rest, and the protrusion of the pseudopodia as the condition of activity, or *vice versâ*. Viewed by the light of the above observations, it is clear that neither state is to be regarded as a resting condition; both are manifestations of activity; both are produced by flowing of the hyaloplasm. Similarly, in the case of muscle, the passage from the contracted to the extended condition can no longer, as is so frequently assumed, be looked upon as a merely passive change of state, but must be regarded, no less than in the case of the passage from the extended to the contracted condition, as produced by flowing of hyaloplasm. In the one case this flows *into* pores of the spongioplasm—this is the condition called contraction, and ordinarily regarded as the active state; in the other case there is a flowing of the hyaloplasm *out of* the pores of the spongioplasm, by which movement the condition of extension is determined. That different chemical and electrical changes accompany, perhaps determine, these different directions of movement is well known. It is also known that the process of extension is influenced by drugs, independently of the action they may exert upon that of contraction (Brunton, Ringer). But whether the chemical and electrical changes, and those produced by drugs, occur in the hyaloplasm, or in the spongioplasm, or in both substances, is a question which, as in the analogous case of the amœboid cell, cannot

at present be decided. The same remark may be made with respect to the question of active participation by the spongioplasm in the production of the movements of the hyaloplasm. It is, however, quite certain from the observation of the movements of the hyaloplasm of pseudopodia, which may actively flow in different directions, even when far removed from the spongioplasm, that it is the hyaloplasm which is to be regarded as the physically active part of protoplasm, and therefore also presumably of muscular substance.

Lastly, there is another form of protoplasmic activity, viz., ciliary motion, which cannot be left out of consideration in any attempt to explain the manner in which the contractile manifestations of protoplasm are produced. On this matter I have no new facts to record, and the suggestion therefore that I have to make must be understood to be a purely theoretical deduction from analogy, and not based upon actual observation. At the same time it does not, so far as I know, stand in contradiction to any known fact. The suggestion is briefly this:—If we suppose that a cilium is a hollow curved extension of the cell, occupied by hyaloplasm, and invested by a delicate elastic membrane, then it must follow that if there be a rhythmic flowing of hyaloplasm from the body of the cell, into and out of the cilium, an alternate extension and flexion of that process would thereby be brought about. The movement would in fact be produced by an action which would be practically the same as that by which the amœboid movements of cells and the contraction and extension of muscle are probably effected. The same result might be got, supposing the cilium to be a straight and not a curved extension of the cell, if the enveloping membrane were thicker (or otherwise less extensible) along one side than along the other. This assumption would also enable one better to account for the spiral direction of the movement of certain cilia; for this form of movement would be produced if the line of lessened extensibility in them were to pass in a corkscrew fashion along the cilium in place of straight along one side, as might be assumed for ordinary cilia.